Talanta 105 (2013) 63–68

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Enzymes immobilized on amine-terminated ionic liquid-functionalized carbon nanotube for hydrogen peroxide determination

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ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form
18 November 2012
Accepted 24 November 2012
Available online 1 December 2012

Keywords:
Functionalization of carbon nanotube
Ionic liquid
Hydrogen peroxide
Cytochrome C
Amperometry

ABSTRACT

We report on a new approach for the electrochemical detection of hydrogen peroxide (H_2O_2) based on Cytochrome C (Cyt c) immobilized ionic liquid (IL)-functionalized multi-walled carbon nanotubes (MWCNTs) modified glass carbon electrode (GCE). Functionalization of multi-walled carbon nanotube with amine-terminated ionic liquid materials was characterized using fourier transform infrared spectroscopy (FTIR), UV–vis spectra, and electrochemical impedance spectroscopy (EIS), and the results showed that the covalent modification of MWCNTs with ILs exhibited a high surface area for enzyme immobilization and provided a good microenvironment for Cyt c to retain its bioelectrocatalytic activity toward H_2O_2. Amperometry was used to evaluate the catalytic activity of the cyt c towards H_2O_2. The proposed biosensor exhibited a wide linear response range nearly 4 orders of magnitude of H_2O_2 (4.0×10^{-8} M–1.0×10^{-4} M) with a good linearity (0.9980) and a low detection limit of 1.3×10^{-8} M (based on $S/N=3$). Furthermore, the biosensor also displays some other excellent characteristics such as high selectivity, good reproducibility and long-term stability. Thus, the biosensor constructed in this study has great potential for detecting H_2O_2 in the complex biosystems.

1. Introduction

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) and a by-product of several oxidative metabolic pathways [1]. The relationship between H_2O_2 concentration and human health has attracted great attention [2], and the determination of H_2O_2 is of practical importance in clinical, environmental, and industrial research. For this reason there has been an increasing interest in the design of reliable H_2O_2 sensors. A number of sensors have been fabricated to determinate H_2O_2 [3–8]. In particular, as biocatalysts, enzymes have a high substrate specificity and reactive efficiency under ambient conditions compared to artificial inorganic catalysts. Nevertheless, the harsh conditions required for chemical reactions limit their applicability because it is difficult to maintain their highly complex molecular configurations [9]. Therefore, a useful strategy to overcome these limitations is to find conductive materials with good biocompatibility.

Carbon nanotubes (CNTs) have been considered as an important class of nanomaterials with outstanding electronic, chemical, and mechanical properties since its discovery in 1991 by Iijima [10]. Recently, a number of research groups have focused on the chemical modification of CNTs through covalent or noncovalent functionalizations [11–16]. These functionalized CNTs modified electrodes exhibited stable electrocatalytical responses towards many important biomolecules such as nicotinamide adenine dinucleotide (NADH) [17], ascorbic acid (AA), dopamine (DA) [18] and cytochrome C (Cyt c) [19]. In addition, many researches focused on CNTs-enzyme-based biosensor [20–22] because CNTs can maintain the bioactivity of enzymes and improve the sensitivity of sensors. However, the major barrier is that coating of the CNTs with an enzyme layer tends to block the electron transport pathway [23] and causes poor dispersion on the CNT surfaces [24], which may greatly limit their application in biosensor systems.

It is widely accepted that the successful synthesis of ILs and CNTs has been proved to be a recent breakthrough in interdisciplinary research because of the unique properties and the striking applications of both kinds of materials [25]. As we know that most biomolecules are charged in biological environments, therefore, IL functionalization of CNTs would provide an alternative method to easily immobilize biomolecules for detection. However, the related studies were few [26,23]. For CNT-based biosensors, there are still some developmental challenges to be addressed. Herein we report our finding that the covalent modification of multiwalled carbon nanotubes (MWCNTs) with imidazolium salt-based ILs provided a good microenvironment

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http://dx.doi.org/10.1016/j.talanta.2012.11.059

for HRP or Cyt c to retain their bioelectrocatalytic activity toward H_2O_2.

2. Experimental

2.1. Reagents

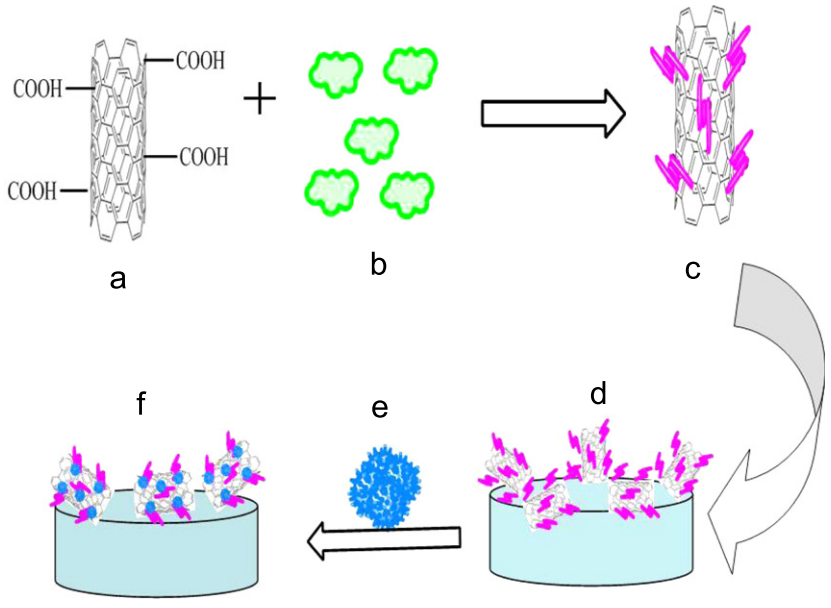
The multi-walled carbon nanotubes (MWNTs) used (diameter: 20–40 nm, length: 1–2 μm, purity: ≥95%) came from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). HRP (> 160 IUmg^{-1}) was from Sigma (USA), and Cyt c came from Shanghai Yuanye biological Co. Ltd (Shanghai, China). Hydrogen peroxide solution (30 wt%) was purchased from Beijing Chemical Reagent (Beijing, China). Bromopropylamine and 1-methylimidazole were obtained from Acros (Beijing, China). And before use, 1-methylimidazole was distilled. PBS (pH 7.0) was prepared by mixing suitable amounts of 0.1 M NaH_2PO_4/Na_2HPO_4. Other chemicals were all of analytical grade, and the solutions were prepared by doubly distilled water.

2.2. Instruments

Amperometric *i–t* curve, cyclic voltammetric (CV) measurements were performed in a conventional three electrodes cell with a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode with a CHI 660c Electrochemical Workstation (Shanghai Chenhua Co., China). UV–vis absorption spectra were taken by absorption mode with a UV-1102 UV–vis spectrophotometer (Shanghai, China). Electrochemical impedance spectroscopy (EIS) experiments were performed on Multi-potentiostat (VMP2, Princeton Applied Research, USA). The working electrode was bare or modified glassy carbon electrode (GCE, d=3.5 mm). All potentials given in this paper were referred to the SCE. Before using, GCE was polished carefully with 0.3, and 0.05 μm alumina slurry to a mirror finish.

2.3. Preparation of Cyt c-MWNT-IL-modified GCE

IL functionalized MWNT (MWNT-IL-Br) was prepared by previously reported method [27]. In general, it was based on an amidation between carboxylic acid functionalized MWNT (MWNT-COOH) and the amine-terminated IL (1-propyl-amine-3-methylimidazolium bromide, IL-NH_2). The IL-NH_2 was prepared by the reaction of 1-methylimidazole (0.02 mol) with 3-bromo-
propylamine (0.02 mol) in 50 mL ethanol under reflux in N_2 atmosphere for 24 h, the ethanol is removed in vacuo and the solid residue dissolved in a minimal quantity of water that is brought to pH 8 by the addition, in small portions, of solid KOH. The product imidazolium bromide is then separated from the KBr byproduct by evaporation of the water, followed by extraction of the residue with ethanol–THF, in which the imidazolium salt is soluble. FTIR verifies the structure and composition of the new IL [28]. MWNT-COOH was prepared by reflux the as-received MWNT in 3 M HCl and H_2O_2. MWNT-IL was prepared by ultrasonicating a solution of 5 mg of the MWNT-COOH, 10 mg of IL-NH_2, and 10 mg of dicyclohexylcarbodiimide (DCC) in 10 mL of dimethylformamide (DMF) for 15 min, and then vigorously stirring at 50 °C for 24 h. Then, unreacted MWNTs were removed by centrifuging. After that MWNT-IL-Br was filtered by nylon membrane with 0.22 μm pores, thoroughly washed with DMF, ethanol and water, separately. Then 18 μL of MWNT-IL-Br aqueous solution (0.05 mg/mL) was dropped on the surface of a GCE. After being dried in air for 24 h, MWNT-IL-Br modified GCE (GCE/MWNT-IL-Br) was thus obtained. Moreover, MWNT-IL-Br modified electrode was immersed in Cyt c solution (1 mg/ml, pH 7.0 PBS) for 2 h at 4 °C. Then Cyt c was immobilized onto the surface of IL-nanohybrid film to form Cyt c-MWNTs-IL/GCE.



a → MWNT-COOH, b → IL-NH_2, c → MWNT –IL–Br,
d → MWNT –IL/GCE, e → Cyt c or HRP, f → Cyt c-MWNT –IL/GCE

Scheme 1. Preparation of Cyt c-MWNT-IL-modified GCE.